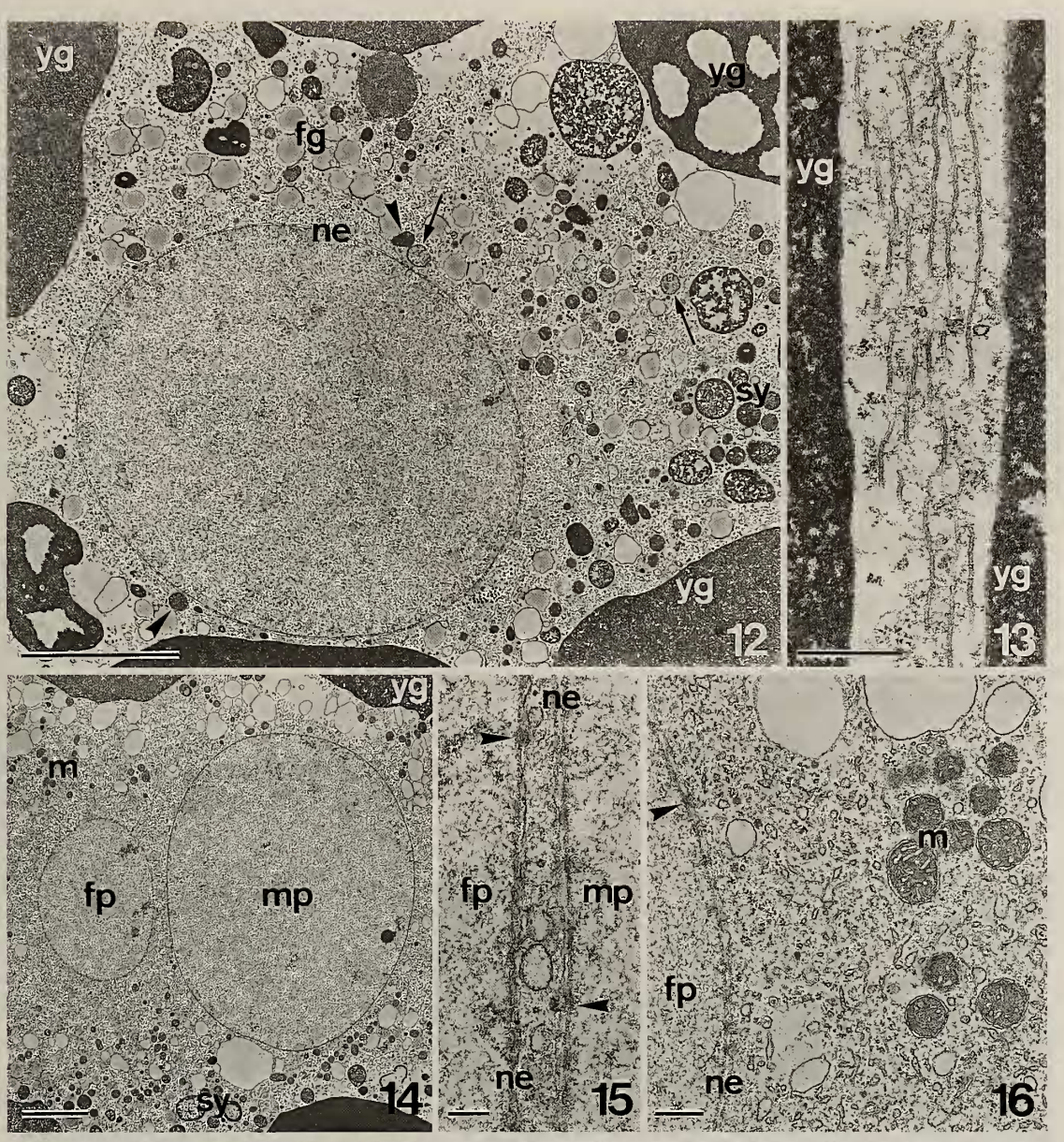

Fig. 12. Electron micrograph showing a male pronucleus and perinuclear cytoplasm. The male pronucleus is enclosed by a complete nuclear envelope (ne) and contains dispersed chromatin. The perinuclear cytoplasm is surrounded by large yolk granules (yg), which are arranged radially (see Fig. 2). Scale bar=5 μm. arrows, weakly electron-dense mitochondria; arrowheads, highly electron-dense mitochondria; fg, fatty granules; sy, small yolk granules.

Fig. 13. Bundles of microtubules between large yolk granules (yg), which are arranged radially around the male pronucleus. Scale bar=0.5 μm.

Fig. 14. Two pronuclei close to one another. The larger pronucleus is probably the male pronucleus (mp) and the other is probably the female pronucleus (fp). Scale bar=5 μm. m, mitochondria; sy, small yolk granules; yg, large yolk granule.

Fig. 15. The nuclear envelopes (ne) of two pronuclei run parallel to each other at a distance of 0.2 μm. The putative female pronucleus (fp) is on the left and the putative male pronucleus (mp) is on the right. Scale ar=0.2 μm. arrowheads, nuclear pores.

Fig. 16. Smooth-surfaced endoplasmic reticulum round the putative female pronucleus (fp). Scale bar=1 μm. arrowhead, nuclear pore; m, highly electron-dense mitochondria; ne, nuclear envelope.

trolled the entrance of sperm into the egg while sperm could pass freely through the chorion. According to Kondo [4], in lycosid spiders, only the thin outer layer of a vitelline membrane, which could not be recognized by light microscopy, was present 30 min after oviposition. The main layer of the vitelline membrane was formed as a very low electron dense layer, containing fibrils, under the outer layer. Kondo suggested that the vitelline membrane bore some resemblance, in terms of its formation, to the fertilization membrane. By contrast, Seitz [9] reported that "the funiculus cells" secreted a precursor component of the vitelline membrane during the first vitellogenic phase in *Cupiennius salei*.

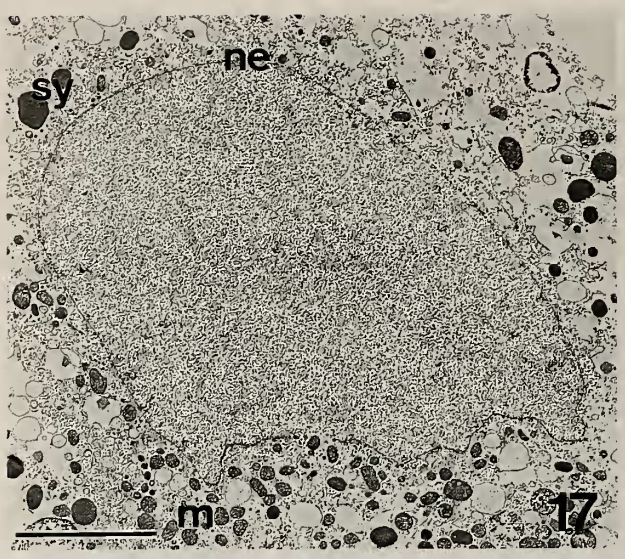

Fig. 17. The zygote nucleus after conjugation. Scale bar = 5 μm. m, mitochondria; ne, nuclear envelope; sy, small yolk granules.

The observations of these authors appear to contradict one another in terms of the origin of the vitelline membrane.

The results of the present investigation strongly support the observations of Kondo [4]. The matrix scattered from the vesicles, by exocytosis, in the space between the cell membrane and the outer layer appeared to form the mucous material and fibrils under the outer layer, and it probably forms the main layer of the vitelline membrane. The formation of the outer layer and that of the main layer may be successive processes that require different materials.

Maturation of the egg

The second maturation division of the first polar body progressed until telophase, but no daughter nuclei were formed. Such division seems similar to that in eggs of *A. tepidariorum* [6]. According to Warren [11], division of the first polar body ceased at metaphase in *Palystes natalius*.

The maturation division of the secondary oocyte was strictly synchronized with respect to that of the first polar body. Montgomery [6] reported that the nucleus of the second polar body was located in the outer radial column of yolk granules. He may have observed the nucleus, as it migrated to the periplasm, immediately after division. Warren [11] described only anaphase in a discussion of the division of the secondary oocyte.

According to Montgomery [6], the chromosomes of the first polar body were not found more than 2 hr after oviposition, and the last time at which the nucleus of the second polar body was observed was 169 min after oviposition. In *A. japonica*, two masses of chromatin of the first polar body and the nucleus of the second polar body were found until 14 hr after oviposition. It is unclear from the present study whether or not polar bodies are eliminated from the egg via the invaginations of the cell membranes.

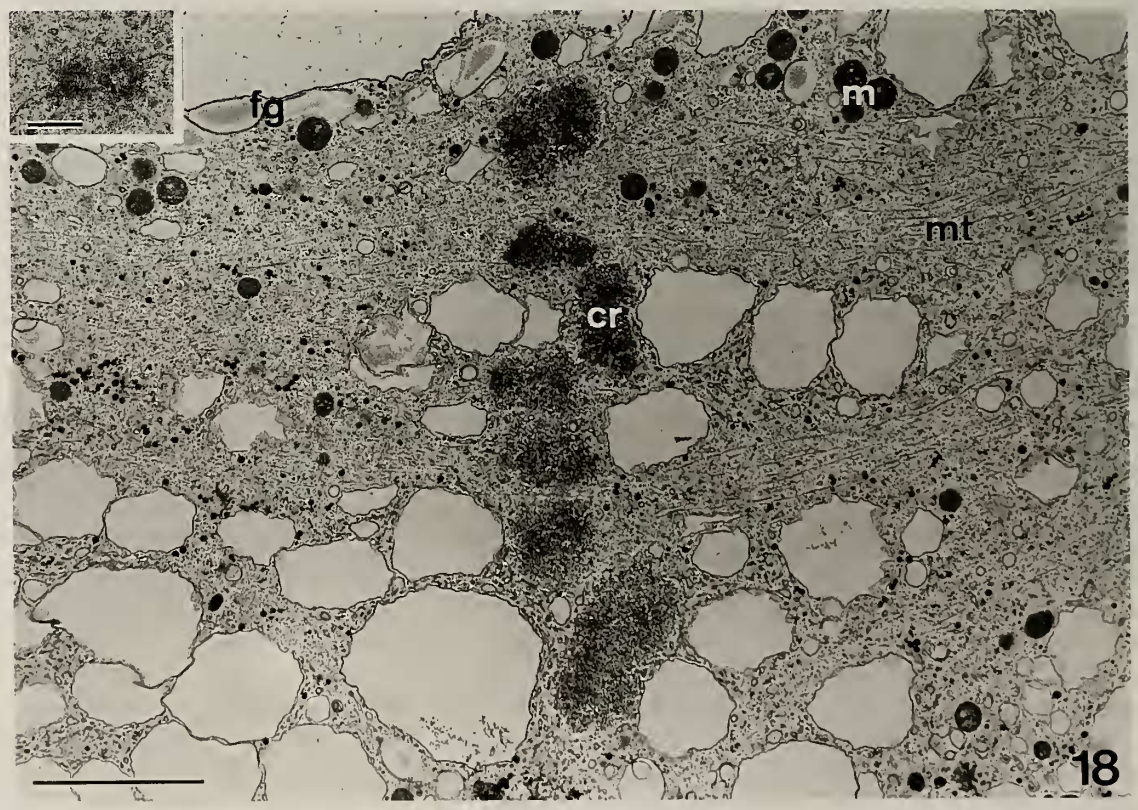

Fig. 18. The first nuclear division at metaphase. Chromosomes (cr) are aligning at the equatorial plane. Scale bar = 5 μm. fg, fatty granules; m, mitochondria; mt, microtubules. Inset. Centrioles at a spindle pole. Scale bar = 1 μm.

Fertilization

The time at which the sperm is incorporated into a spider's egg remains unknown. Montgomery [6] reported that eggs accepted a sperm nucleus that was located about halfway between the periphery and the center of the egg at oviposition, while Warren [11] and Rempel [8] suggested that fertilization occurs after oviposition. The present investigation supports the evidence reported by Montgomery. From the location of the sperm nucleus in the newly laid egg, the incorporation of sperm in the ovarian cavity can be postulated.

The sperm nucleus was observed as a mass of condensed chromatin, and it was not enclosed by a nuclear envelope. The nuclear envelope may be broken down at the time of incorporation of the sperm. Mitochondria that had a weakly electron-dense matrix may have been of paternal origin. The large yolk granules surrounding the perinuclear cytoplasm were arranged radially from immediately after oviposition. This arrangement of large yolk granules may be caused by the radially distributed cytoplasm that contains numerous bundles of microtubules that radiate from the perinuclear cytoplasm. Holm [2] called the cytoplasm that connects the perinuclear cytoplasm to the periplasm "plasm threads", and he suggested that they allow cleavage nuclei to migrate toward the periphery of the egg. By contrast, Kondo [4] suggested that the migration of the cleavage nuclei might be related to the decomposition of yolk granules and to motion of the cell membrane, and he also suggested that the plasm threads, which he described as a protoplasmic reticulum, only lay between the perinuclear cytoplasm and the periplasm. He would not have detected any bundles of microtubules because he prepared his samples by fixation at 0–4°C. Microtubules tend to be depolymerized at such low temperatures [10]. We suggest that the radial bundles of microtubules are equivalent to the plasm threads and that they play an important role in the migration of the sperm nucleus, accompanied by the perinuclear cytoplasm, to the center of the egg.

In previous studies of many spiders [1–3, 5, 7, 12], a nucleus has been noted at the center of the egg immediately after oviposition. This nucleus may have been the male pronucleus. The sperm nucleus arrives at the center of the egg and then develops into the male pronucleus. The chromatin ceases to be condensed, and fragments of the original nuclear envelope may be reconstructed as the complete nuclear envelope. The female pronucleus, generated by the second maturation division, should then move toward the center of the egg, in which the male pronucleus is now located. However, no migrating female pronucleus was observed in the present study.

In *A. japonica*, the male and female pronuclei lie close to one another for about one hour before conjugation, and each increases in size. Montgomery [6] stated that the larger nucleus was certainly the sperm nucleus because it was similar in volume to the supernumerary sperm nuclei.

Most of the mitochondria with a highly electron-dense matrix may have immigrated with the female pronucleus. While rod-shaped mitochondria were observed in the periplasm (Suzuki and Kondo, unpublished data), they were hardly ever observed in the perinuclear cytoplasm.

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